Remarks/Arguments:

This is a reply to the office action of July 21.

Although a valid priority reference to provisional application 60/510231 has been made, the examiner stated that "A claim of priority cannot be based on said application" on the ground it does not support claim 1. We submit that a priority application need not support every claim in order to be a priority application; it may be that it supports some claims and not others; for those, the effective filing date may be different. We understand the examiner to mean claim 1 is not entitled to priority from the provisional application, and address the support issue further below. Moreover, claim 1 has been substantially amended above.

We have amended the abstract to comply with U.S. rules.

The missing sequence ID's referred by the examiner are two sequences in paragraph [0132] of the published application which were not included in the sequence listing. We have accordingly amend the sequence listing by inserting two entries, and we have amended paragraph [0132] accordingly. No more missing sequence IDs were found in the application. An amendment sequence listing in text format is filed herewith.

We traverse the objection raised (35 USC 112, first paragraph) by the examiner. It is true that the identification of new split-protein sensors is difficult since selection of suitable fragmentation sites depends on various factors and that pre-selected fragmentation sites might not work as expected. This is exactly the problem that the claimed invention overcomes. The invention provides a system where a large library of randomly created fragmentation sizes in a reporter protein is generated and where this library is then subsequently screened for fragment pairs that can reconstitute a native-like and active protein *in vivo*. The invention therefore provides an efficient method to identify such fragments, without having to first identify possible suitable fragments through sequence or conformational analysis. Thus, the skilled artisan now for the first time may well predict the operability of the invention for any

protein, as required by Fed. Cir. in *In re Curtis,* 354 F.3d 1347, 1358, 69 USPQ2d 1274, 1282 (Fed. Cir. 2004)

The examiner concluded that the specification and claims are based on prophetic statements. We strongly disagree. The generation of libraries with random sequences is well known in the art. An exemplary method to generate such a library is given in the specification in paragraph [0024]. This exemplary method uses standard DNA manipulation techniques known in the art, such as PCR amplification, PCR overlap extension and molecular cloning techniques. These techniques are all very well established and optimization of the protocols is routine work for a person skilled in the art. A person skilled in the art is also able to create such a library for a large number of other genes than Trp, since the standard DNA manipulation techniques cited above are well established in the art.

In response to the rejections under 35 USC 112, second paragraph, we have amended claim 1 to comply with the statute. In particular, step (c) has been replaced by the features of claim 4, specifying in more detail how the screening for suitable fragment pairs is done.

With respect to point 2, we disagree with the examiner's opinion, since the terms "first subdomain", "complementary second subdomain" are defined in paragraph [0010] and the terms "first subsequence" and "complementary second subsequence" are defined in [0011].

The inventive method aims to identify suitable fragment pairs in different reporter proteins and the subdomains and subsequences refer to the DNA sequences of these, so it is not possible to specify a concrete sequence. The inventive idea behind the method is exactly to be able to apply it to any reporter protein and hence the subdomains and subsequences will vary depending on which reporter protein this method is applied to.

DNA libraries are well known in the art and a skilled artisan understands that each individual in the library contains at least one vector coding for the desired sequence or sequences. Generation of vectors for the library of the method of the present invention is explained in [0024]. From this it is clear that each individual of the library comprises two subsequences

coding for two subdomains of the reporter protein which were previously generated through PCR and cloning techniques.

The unclear formulation "and/or" is no longer present in amended claim 1.

The term "close proximity" has been altered to "close spatial proximity so as to allow reconstitution of both first and second subdomains into an active protein" so as to more clearly specify the measure of the required distance. 6.

In claim 3, "in one and the same expression vector" has been replaced by "in one expression vector"

In claim 8, "...the transcription..." has been clarified by reciting "...the transcription...into a polypeptide". Transcription of a gene into a polypeptide is well known and therefore this new formulation should be unambiguous.

In claim 10, "the wild type length" has been changed to "the wild type length of TRP1"; "the original N- and C-termini" has been clarified by reciting "the original N- and C-termini of TRP1"; "the vector" has been replaced by the "the expression vector" which is defined in the third step of the method of claim 10; and "the zippers" have been replaced by "the leucine zippers" which are defined in the same step of claim 10.

The rejections based on anticipation by Balint et al. (US 20040018317) are respectfully traversed. Balint et al discloses a method of identifying interaction between proteins or peptides which have been genetically or chemically conjugated to the break point termini of fragment pairs of a Class A β-lactamase (TEM-1 of E. coli) as reporter protein. Interaction of these proteins or peptides will allow the lactamase to reassemble into an active protein thus making the identification of such interactions possible. Balint also discloses the generation of a protein-protein interaction library based the use of the lactamase as reporter protein.

In contrast according to the present invention, the sequence of a known reporter protein is fragmented into two subse quences of random lengths and each subsequence is then coupled to a nucleotide sequence coding for proteins or peptides which are known to interact with each other resulting in sequences coding for two fusion proteins. The method as described in Balint relies on subdomains of a reporter protein with a defined length and which are known to be able to reconstitute an active-like protein. These fragments are coupled to two proteins or peptides to find out whether these interact with each other.

Balint proposes at [0037] a method to identify functional fragment pairs of a reporter protein. The major difference to the method of the present invention is that in Balint suitable break points within solvent exposed loops have to be selected beforehand following a tertiary or secondary structure analysis. The method of the present invention does not necessitate any analyis of protein structure, but enables the generation of a library with random fragment pairs of a reporter protein. This library may then be screened for fragment pairs which reconstitute the function of the reporter protein in vivo. The present method considerably speeds up the procedure to find suitable fragment pairs and does also enable the generation of split-protein reporter systems from proteins where the structure has not yet been analyzed. Johnson et al (PNAS) similarly describes a method to determine protein-protein interactions by the way of restoration of ubiquitin mediated degradation. Ubiquitin is split in two fragments, which will reconstitute a native-like protein structure only when fused to two proteins or peptides interacting with each other. Johnson does not describe any method which is suitable to identify suitable reporter protein fragments. On the contrary, the method described relies on reporter protein fragments of a known length which are fused with proteins or peptides to be screened for interaction. The method of the claimed invention however relies on protein or peptides which are known to interact to screen a library with fragments of a reporter protein having random lengths to identify fragment pairs being able to reconstitute a native-like protein.

Similarly, Luger et al (Science) and Eder et al (Biochemistry) also rely on specific selection of fragmentation sites. Therefore, in contrast to the claimed invention, both Luger and

Eder do not disclose the use of randomly created subsequences of the DNA encoding for subdomains of the protein. Therefore, claim 1 is novel over these references. Neither of these publications proposes a system to generate random length fragments of a protein or the generation of a library.

As for Tafelmayer et al. (Chemistry and Biology), this document was published in 2004 (see first page of the document) by the inventors less than a year before the filing date of the international application (October 8, 2004). Therefore, that document is not prior art under 35 U.S.C. 102(b), even if the prior U.S. provisional application's date is disregarded.

We believe all the claims now presented are patentable over the prior art of record, and that this application is condition for allowance.

Respectfully submitted,

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October 21, 2009